Rejection under 35 U.S.C. §112, first paragraph

The rejection of claims 1-31 under 35 U.S.C. §112, first paragraph, has been withdrawn. Applicants acknowledge with gratitude the withdrawal of this rejection.

Rejection under 35 U.S.C. §112, second paragraph

Examiner has withdrawn the rejection of claim 31, based on the recitation of "in whole or in part". Claim 31 was amended, in the Preliminary Amendment filed December 26, 1997, to delete the phrase "in whole or in part". Applicants acknowledge with gratitude the withdrawal of this rejection.

The Office has maintained the rejection of claims 1-23 and 31 under 35 U.S.C. §112, second paragraph for use of the term "substantially". Independent claims 1 and 18 are directed to methods which "substantially prevent" depletion of non-autologous hematopoietic cells in an animal; claim 19 is directed to a non-human mammal having a decreased level of endogenous macrophages sufficient to prevent "substantial depletion" of non-autologous hematopoietic cells. Claim 31, which is directed to a method of improving engraftment efficiency, does not include the term "substantially". Accordingly, this rejection, as it pertains to claim 31, is moot.

The Office concludes from reading page 11 of the specification that "only 1-10% of the administered cells remain after several days, and this does not correlate with the 'substantial prevention of depletion', as claimed, since 90-99% of the cells have been depleted." Applicants disagree and respectfully submit that the Office has misunderstood the description in the specification. The Office apparently believes that the percent of cells remaining of those that were originally introduced is the parameter being measured. This is not correct. The human hematopoietic cell content of the mouse peripheral blood cells, i.e., the percent of the peripheral blood cells remaining which are human (non-autologous), is the parameter being measured. As shown in Example 8 and Figure 3, the percent of peripheral blood cells which were human was initially less than 1%. Mice treated with phosphate-buffered saline (PBS) did not demonstrate a significant change in levels of human cells in the periphery following injection, while five of eight mice treated with DMDP in liposomes demonstrated a rise in human cells in the periphery 2-5 days post injection (days 18 and 21 in Figure 3A). (bridging paragraph, pp. 22-23). Clearly,

what is being measured is the percentage of peripheral blood cells that are human, not the percent of cells remaining that were originally present.

Applicants believe that all the rejections on the grounds of indefiniteness have been addressed and that the above remarks overcome the rejections. Therefore, it is respectfully requested that these rejections under 35 U.S.C. §112, second paragraph, be withdrawn.

Rejections under 35 U.S.C. §103

The Examiner has withdrawn the rejection of claims 1-17 and 19-23 under 35 U.S.C. §103, as allegedly unpatentable over Aldrovandi taken with Pinto. The Examiner has further withdrawn the rejection of claim 18 under 35 U.S.C. §103, as allegedly unpatentable over Aldrovandi taken with Pinto as applied to claims 1-17 and 19-23 and further in view of Bernstein. The Examiner has further withdrawn the rejection of claims 24-30 under 35 U.S.C. §103, as allegedly unpatentable over Berenson and Baum taken with Pinto. Finally, the Examiner has withdrawn the rejection of claim 31 under 35 U.S.C. §103, as allegedly unpatentable over Baum taken with Pinto.

However, the Office has applied new grounds of rejection of the claims over the combination of the previously cited art combined with newly cited art.

Specifically, claims 1-17 and 19-23 stand rejected under 35 U.S.C. §103 as allegedly unpatentable over Aldrovandi taken with Pinto, Pflumio and Kuby (Immunology, 1992, W.H. Freeman and Co., New York; hereinafter "Kuby"). Claim 18 is rejected under 35 U.S.C. §103 as allegedly unpatentable over Aldrovandi and Pinto as applied to claims 1-1 and 19-23 and further in view of Bernstein. Claims 24-30 are rejected under 35 U.S.C. §103 as allegedly unpatentable over Berenson and Baum taken with Pinto, Pflumio and Kuby. Claim 31 is rejected under 35 U.S.C. §103 as allegedly unpatentable over Baum taken with Pinto, Pflumio and Kuby.

1. Rejection over Aldrovandi taken with Pinto, Pflumio and Kuby.

Applicants traverse the rejection of claims 1-17 and 19-23 under 35 U.S.C. §103 as allegedly obvious over Aldrovandi taken with Pinto, Pflumio and Kuby.

Independent claim 1 is directed to a method of preventing depletion of non-autologous hematopoietic cells comprising decreasing the number of endogenous macrophages to a level sufficient to substantially prevent depletion of the non-autologous hematopoietic cells. Claims 2-17 depend variously from claim 1. Independent claim 19 is directed to a non-human mammal comprising human hematopoietic cells, wherein the mammal contains a decreased level of endogenous macrophages sufficient to prevent depletion of non-autologous hematopoietic cells. Claims 20-23 depend from claim 19.

Aldrovandi teaches the use of SCID-hu mice as an *in vivo* system for studying HIV pathology. SCID-hu mice were generated by implanting human fetal liver and human fetal thymus into SCID mice; these mice were then infected with HIV-1. It was shown that HIV-1 could infect the $CD4^+$ human cells, which resulted in a depletion of these cells. The Office correctly points out that "the reference fails to disclose decreasing the number of endogenous macrophages to a level to prevent depletion of the non-autologous hematopoietic cells." The focus of the study described by Aldrovandi apparently is not related to efforts to prevent depletion of non-autologous hematopoietic cells, since Aldrovandi does not suggest prevention. Rather, Aldrovandi points toward the depletion of the non-autologous cells, i.e., the implanted $CD4^+$ human cells, by HIV-1 infection as a positive result, since it showed that "HIV-1 infection of the SCID-hu mouse reproduces key aspects of HIV-1 pathology in man and may be an important small animal model to study HIV-1-induced pathogenesis *in vivo*." (p. 735, col. 1, lines 1-4).

The Office argues (p. 3, paragraph 4) that Aldrovandi is relevant, since "the 'rapid depletion of non-autologous hematopoietic cells' is not seen to be different than the GVHD or other types of tissue rejection which take place upon transplantation." The purpose of this argument is unclear, since Aldrovandi does not address the problem of GVHD or any other type of tissue rejection.

The Office goes on to state that Pinto, Pflumio and Kuby cure the deficiency. The Office states that Pinto discloses that administration of DMDP encapsulated in liposomes and administered intravenously will deplete splenic and liver macrophages. Pinto indeed discloses the depletion of macrophages by DMDP. However, Pinto discloses the effects of DMDP on

antimicrobial resistance, not on depletion of non-autologous hematopoietic cells. The Office further states that Pflumio discloses that adult SCID mice, while deficient in specific T and B cell immunity, still possess normal levels of non-specific activities, such as natural killer (NK) cells and macrophages. Pflumio does not indicate, however, that macrophages have any involvement in depletion of non-autologous cells. Rather, Pflumio appears to implicate NK cells, as shown in the discussion of the possible reasons for the differences between adult and newborn SCID mice.

> "Differences between adult and newborn recipient mice could be due to the increased immunodeficiency in newborn mice, a more stimulatory microenvironment, or simply achieving a higher cell dose/body wt. Evidence that the level of immunodeficiency affects human cell engraftment comes from a recent report showing that adult SCID mice pretreated with anti-asialo GM1 before human PBL transfer support higher levels of T cell engraftment than untreated transplanted mice (19) suggesting that natural killer cells play a role in regulating the engraftment of the human cells." [emphasis added; reference 19 is Murphy et al. (1992) *Eur. J. Immunol.* 22:1421]

Again, Pflumio neither teaches nor suggests that macrophages are the cell type responsible for reducing the levels of non-autologous cells. If anything, Pflumio might have suggested to one of skill in the art that NK cells should be the target of efforts to prevent depletion of non-autologous cells.

The Office states (Paper No. 20, p. 3, para. 3) that "Applicants have argued that there is no teaching directed to the role of macrophages in the engraftment response and in transplantation generally." The Office has provided the Kuby reference, which the Office contends demonstrates that macrophages were known to play a role in graft rejection.

The Office states that Kuby discloses that macrophages are involved in allograft rejection. In fact, Kuby merely states that a hallmark of graft rejection is an influx of T lymphocytes and macrophages into the graft. (p. 492, paragraph 2, lines 7-9). Infiltration into the graft does not necessarily imply a direct involvement in depletion of the engrafted tissue. Macrophages have a variety of functions, which include phagocytosis of exogenous antigens such as whole microorganisms, insoluble particles, injured and dead host cells, cellular debris

and activated clotting factors; antimicrobial activity toward phagocytosed microorganisms; antitumor activity; and antigen processing and presentation. (see, for example, Immunology, 2nd ed., J. Kuby, ed. (1994), W.H. Freeman, New York, pp. 62-66, copy attached). That macrophages infiltrate an allograft would perhaps not have been surprising to one of skill in the art, since macrophages phagocytose dead and injured cells and cellular debris, and would have been present at a site of tissue injury where such cells were present.

Indeed, Kuby discusses the role of cell-mediated immunity, and suggests that T cells are the primary culprits in graft rejection, since "nude mice, which lack a thymus and consequently lack functional T cells, were found to incapable of allograft rejection". (bridging paragraph, pp. 488-489). Further on, Kuby states that "Analysis of the T-cell subpopulations involved in allograft rejection has implicated both $CD4^+$ and $CD8^+$ populations." Clearly, then, one skilled in the art, upon reading Kuby, would not be motivated to deplete macrophages in order to prevent allograft rejection.

As noted in the specification, the role of macrophages included phagocytosis of particles (p. 2, lines 32-35) and the findings were that macrophages in the mononuclear phagocytic system play an important role in clearance of non-autologous hematopoietic cells and that elimination of endogenous macrophages results in the ability of non-autologous hematopoietic cells to circulate and survive in the periphery of host animals (p. 3, lines 26-31). As noted above, Kuby suggests that T cells are the primary culprits in graft rejection, since nude mice, which lack a thymus and consequently lack functional T cells, were found to be incapable of allograft rejection. In this context, it is important to note that nude mice, while lacking functional T cells, still have functional macrophages, yet do not reject allografts. This is additional evidence that it was an unexpected result that reducing macrophage activity would prevent clearance of non-autologous hematopoietic cells.

The Office mentions that Kuby implicates macrophage involvement in graft-versus-host disease (GVHD). However, the phenomenon being examined in the instant application is not GVHD, but the destruction of engrafted cells by the host. The citation of the discussion of GVHD thus appears to be irrelevant to the claimed invention.

The Office concludes that "it would have been obvious to one of ordinary skill to modify the method of Aldrovandi by treating the SCID-hu mice with DMDP in order to kill the endogenous macrophages", "as suggested by Pflumio and Kuby". Pinto does not address and therefore does not provide a solution to the problem of clearance of non-autologous hematopoietic cells. Aldrovandi does not address the problem of depletion of non-autologous hematopoietic cells by the host. Pinto does not suggest the use of DMDP in prevention of clearance of non-autologous hematopoietic cells. Neither Pflumio nor Kuby teaches that macrophages are the mediators of the depletion. Therefore, one skilled in the art would not, indeed could not, be motivated by the combination of these references to arrive at the invention defined by claims 1-17 and 19-23.

Furthermore, the Office has failed to provide an explanation why, if, as the Office contends, the invention defined by claims 1-17 and 19-23 is obvious, the Marcus reference and the Shpitz reference show that, well after the priority date of the present application, one did not know which cells were responsible for the depletion of non-autologous cells.

The Office (p. 4, para. 2) argues that "Applicants' arguments regarding the teachings of Shpitz are not persuasive since Shpitz uses monoclonal antibodies and Kuby establishes the role of macrophages in graft rejection." First, it is unclear why the use of monoclonal antibodies would render the argument unpersuasive. Second, Shpitz in fact used a rabbit polyclonal antibody to NK cells, which resulted in reduction of NK cells. This treatment, together with irradiation, "resulted in efficient engraftment of human PBLs into the spleen of the SCID mouse at very high levels." (Shpitz at 9. 2, col. 2, lines 10-12). Third, as discussed above, Kuby does not establish the role of macrophages in graft rejection.

Claim 2 depends from claim 1 and recites that non-autologous hematopoietic cells are injected into an animal. Claim 15 depends from claim 7 and recites that non-autologous hematopoietic cells are injected into the immunocompromised animal. Regarding claims 2 and 15, the Office argues that the "administration of autologous cells by administration is not different than administration by transplantation", "since the stimulation of leukocytosis by DMDP and simultaneous depletion of macrophages by DMDP would still result." This rejection

is unclear, since both claims 2 and 15 recite administration by injection of non-autologous hematopoietic cells.

Regarding claim 6, the Office is of the opinion that "the use of mice having naturally occurring low levels of macrophages would be obvious to one of ordinary skill since the purpose of the use of DMDP is to reduce the levels of macrophages." Applicants respectfully submit that the Office is under an apparent misapprehension regarding the invention. Claim 6 depends from claim 1 and is directed to a method for genetically decreasing the number of endogenous macrophages. The invention as described in claim 1 is to a method of preventing depletion in an animal comprising decreasing the number of endogenous macrophages. Claim 1 does not include limitations as to how one effects the decrease. Indeed, in the specification, it is disclosed that depletion of endogenous macrophages "can be effected by any method known in the art, including, but not limited to, the transgenic elimination or inactivation of macrophages ...". Claim 6 is directed to the genetic reduction in endogenous macrophages. Genetic reduction is just one of several means disclosed for reducing endogenous macrophages. Treatment with DMDP is another, separate method.

Claim 9 is a dependent claim directed to a method for preventing depletion of non-autologous hematopoietic cells in an immunocompromised human infected with human immunodeficiency virus, comprising decreasing the number of endogenous macrophages. Claim 17 is a dependent claim directed to a method of preventing depletion of non-autologous hematopoietic cells in a human, wherein the non-autologous cells are injected. Regarding claims 9 and 17, the Office states that Aldrovandi discloses that the SCID-hu mouse infected with HIV-1 may be an important small animal model to study HIV-1-induced pathogenesis *in vivo*, and that Pinto teaches that macrophage depletion interferes with the immune response and also stimulates lymphocyte production. In fact, Pinto teaches that DMDP, in addition to resulting in depletion of macrophages, resulted in leukocytosis. However, leukocytosis should not be confused with lymphocyte production. Indeed, as stated in Pinto, "The mechanism involved in the peripheral blood leukocytosis caused by DMDP liposome treatment requires further study. ... The leukocytosis may therefore be related to an outflow of the large circulating pool of lymphocytes and PMN that is contained within the spleen and liver...". (p. 582, col. 1, paragraph 1). Thus, it

is suggested that the observed leukocytosis results from mobilization of leukocytes from organs, not increased lymphocyte production. Moreover, Pinto notes that DMDP treatment causes a transient decrease in NK cells. Nowhere does Pinto suggest the use of DMDP to deplete macrophages for the purposes of reducing clearance of non-autologous hematopoietic cells. If anything, Pinto teaches away from the claimed invention by suggesting that leukocytes are mobilized, which, given the teaching in Kuby that CD4+ and CD8+ populations are involved in allograft rejection, might have suggested to one of ordinary skill in the art that treatment with DMDP could actually promote depletion of non-autologous cells. Given the teachings in the art that T cells mediate allograft rejection, one of ordinary skill in the art would not have been motivated to treat animals with a compound that effected the mobilization of leukocytes, which include T cells.

According to the Office, it would have been obvious to one of skill in the art to apply the method of Aldrovandi modified by Pinto to humans having HIV infection. The Office states that "Transplanted autologous T cells, or bone marrow or PBL would not be rejected in view of the teachings of Pinto [that] DMDP interferes with the immune response." This rather broad logical leap from depression of an immune response to microorganisms to lack of rejection of transplanted cells does not appear to have a clear factual basis. Interference with the immune response to a microbial pathogen doesn't necessarily result in alteration of all aspects of an immune response. For example, nude mice, which lack functional T cells, still have macrophages and B lymphocytes and are capable of carrying out immune functions that do not depend upon functional T cells. (See, for example, Fundamental Immunology (1989) W. Paul, ed. Raven Press, pp. 1047-1048, copy attached).

Claims 10 and 11 are dependent claims and are directed to methods of preventing depletion of non-autologous hematopoietic cells in an animal which is immunocompromised due to radiation therapy and chemotherapy, respectively, the method comprising decreasing the number of endogenous macrophages. Regarding claims 10 and 11, the Office states that "ablation of the immune system to deplete the host of immune responding cells would be obvious in view of the teachings of Aldrovandi that the host must be immunocompromised in order to allow transplantation of autologous tissue." Again, Applicants respectfully submit that

the Office is under an apparent misapprehension regarding the invention as recited in claims 10 and 11. The Office is apparently of the belief that the invention involves intentional ablation of the host immune system. This is incorrect. The invention defined in claims 10 and 11 is a method of treating an animal that is immunocompromised due to radiation therapy or chemotherapy, the method comprising depletion of host macrophages so as to allow the host to accept non-autologous hematopoietic cells. Introducing non-autologous hematopoietic cells into an immunocompromised animal has the potential to save the life of the animal by providing the animal with immune functions which it may lack by virtue of its being immunocompromised.

Claim 19 is directed to a non-human mammal comprising human hematopoietic cells, wherein the mammal contains a decreased level of endogenous macrophages sufficient to prevent substantial depletion of non-autologous hematopoietic cells. Claims 20-23 depend from claim 19. Regarding claims 19-23, the Office states that the combination of Aldrovandi taken with Pinto, Pflumio and Kuby renders obvious the non-human mammal since Aldrovandi discloses SCID-hu mice containing human hematopoietic cells and Pinto discloses the use of DMDP to inactivate macrophages. The Office further states that Aldrovandi, Pflumio and Kuby provide the motivation to combine the references. Aldrovandi discloses a SCID-hu mouse system which allows HIV-1 infection of human cells to be studied. The Office contends that Pflumio discloses that "macrophages could interfere with human cell engraftment" and suggests using newborn mice, and that Kuby "discloses that macrophages are known in the art to be involved in graft (bone marrow) rejection." Applicants disagree. As discussed above, neither Pflumio nor Kuby teaches that macrophages are involved in graft rejection. Aldrovandi discloses a SCID-hu mouse system for studying HIV-1 infection of human cells, but neither teaches nor suggests the present method of preventing depletion of non-autologous hematopoietic cells by decreasing the number or activity of host macrophages. Similarly, Pinto does not address the problem of clearance of non-autologous hematopoietic cells. Accordingly, the combination of these references could not have provided the motivation to one of ordinary skill in the art to arrive at the invention as defined in claims 19-23.

2. Rejection over Aldrovandi, Pinto, Pflumio, Kuby and Bernstein

Claim 18 is rejected under 35 U.S.C. §103 as allegedly unpatentable over Aldrovandi and Pinto as applied to claims 1-17 and 19-23 above, and further in view of Bernstein. Independent claim 18 is directed to a method of treating an immunocompromised animal comprising administering to the animal non-autologous hematopoietic cells and decreasing endogenous macrophages.

The Office states that Bernstein discloses that macrophage growth factors such as GM-CSF, M-CSF and IL-3 may enhance HIV replication in mononuclear phagocytes and that this suggests that activation of replication pathways in these cells may also be associated with viral stimulation. The Office goes on to state that "it would have been obvious to one of ordinary skill then to inactivate macrophages as a method of treatment in order to abolish viral replication." Applicants disagree. First, Bernstein discloses that activation of human monocyte-derived macrophages infected with HIV-1 in vitro demonstrated a decrease in viral p24 release after incubation with LPS. This would appear to be at odds with the Office's contention that one of ordinary skill in the art would have been motivated to inactivate macrophages as a means to reduce viral replication. Second, even if it were true, as the Office contends, that "it would have been obvious to one of ordinary skill then to inactivate macrophages as a method of treatment in order to abolish viral replication", the invention as defined by claim 18 is not directed toward a method of abolishing viral replication. It is directed toward a method which comprises reducing endogenous macrophages to prevent depletion of non-autologous hematopoietic cells in an immunocompromised animal. The Office has contended (p. 5, para. 1) that the invention "is directed to treating the HIV condition in a hu-SCID mouse model." This is not correct. As stated above, the invention is not directed to a method for abolishing HIV infection. The immune system of an immunocompromised animal can be at least partially restored by transfusion with allogeneic hematopoietic cells. Immunocompromised animals still retain the capacity to deplete non-autologous hematopoietic cells. Therefore, the method is directed to administering to an immunocompromised animal non-autologous hematopoietic cells in order to at least partially restore a functional immune system, and to decreasing endogenous macrophages so as to prevent substantial depletion of the non-autologous hematopoietic cells.

The Office states that "a method of treating an immunocompromised animal was within the ordinary skill in the art at the time the claimed invention was made." Applicants respectfully point out that this does not indicate that the claimed invention was obvious. "Within the ordinary skill in the art" implies "within the technical capabilities of one of ordinary skill in the art". That the claimed invention was within the technical capabilities of one of ordinary skill in the art is not in dispute.

Given that Bernstein could not have motivated one of ordinary skill in the art to inactivate macrophages, and that, as discussed above, neither Pflumio nor Kuby teaches that macrophages mediate graft rejection, it could not have been obvious to one of ordinary skill in the art to combine Aldrovandi, Pinto, Pflumio, Kuby and Bernstein to arrive at the invention as defined by claim 18.

3. Rejection over Berenson, Baum, Pinto, Pflumio and Kuby.

Claims 24-30 are rejected under 35 U.S.C. §103 as allegedly unpatentable over Berenson and Baum taken with Pinto, Pflumio and Kuby.

Claim 24 is an independent claim directed to a method of restoring hematopoietic cells to an immunocompromised human, comprising the steps of administering human peripheral blood cells in conjunction with decreasing endogenous macrophages. Claims 25-30 depend variously from claim 24.

The Office correctly notes that Berenson and Baum do not disclose decreasing endogenous macrophages. The Office goes on to state that Pinto, Pflumio and Kuby cure the deficiency. However, for the reasons stated above, Pflumio and Kuby cannot cure the deficiency, as neither reference teaches that macrophages mediate graft rejection. Furthermore, as discussed above, Pinto does not address the problem of clearance of non-autologous hematopoietic cells. Accordingly, these references could not have rendered the claimed invention, as defined by claims 24-30, obvious.

4. Rejection over Baum, Pinto, Pflumio and Kuby.

Claim 31 is rejected under 35 U.S.C. §103 as allegedly unpatentable over Baum taken with Pinto, Pflumio and Kuby.

Claim 31 is directed to a method of improving engraftment efficiency for transplantation of a population of non-autologous hematopoietic stem cells (HSC's), comprising ablating the endogenous HSC population and transplanting non-autologous HSC's in conjunction with decreasing endogenous macrophages.

The Office states that it would have been obvious to one of ordinary skill to modify the method of Baum by using DMDP in view of the teachings of Pinto. Applicants disagree. Baum teaches transplantation of human bone marrow into SCID mice and identification of a human HSC population. Baum does not address the problem of clearance of non-autologous HSC's. Nor does Pinto address the problem of clearance of non-autologous HSC's. Accordingly, one of ordinary skill in the art could not have been motivated to arrive at the invention as defined by claim 31 by combining Baum and Pinto. Since neither Kuby nor Pflumio teaches that macrophages mediate clearance of non-autologous HSC's, these references do not cure the deficiency.

Applicants believe that all the rejections on the grounds of obviousness have been addressed and that the above arguments overcome the rejections. Therefore, it is respectfully requested that these rejections under 35 U.S.C. §103 be withdrawn.

III. CONCLUSIONS

Applicants submit that the above discussion is fully responsive to all grounds of rejection set forth in the Office Action. In view of the comments above, Applicants respectfully request that all outstanding rejections be withdrawn, and that the pending claims, as amended, be allowed. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned attorney at (650) 813-5730.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicant petitions for

any required relief including extensions of time and authorizes the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket no. 202962001301. However, the Assistant Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Respectfully submitted,

Dated: March 19, 1998

By: _____

Antoinette F. Konski
Registration No. 34,202

Morrison & Foerster LLP
755 Page Mill Road
Palo Alto, California 94304-1018
Telephone: (650) 813-5600
Facsimile: (650) 494-0792